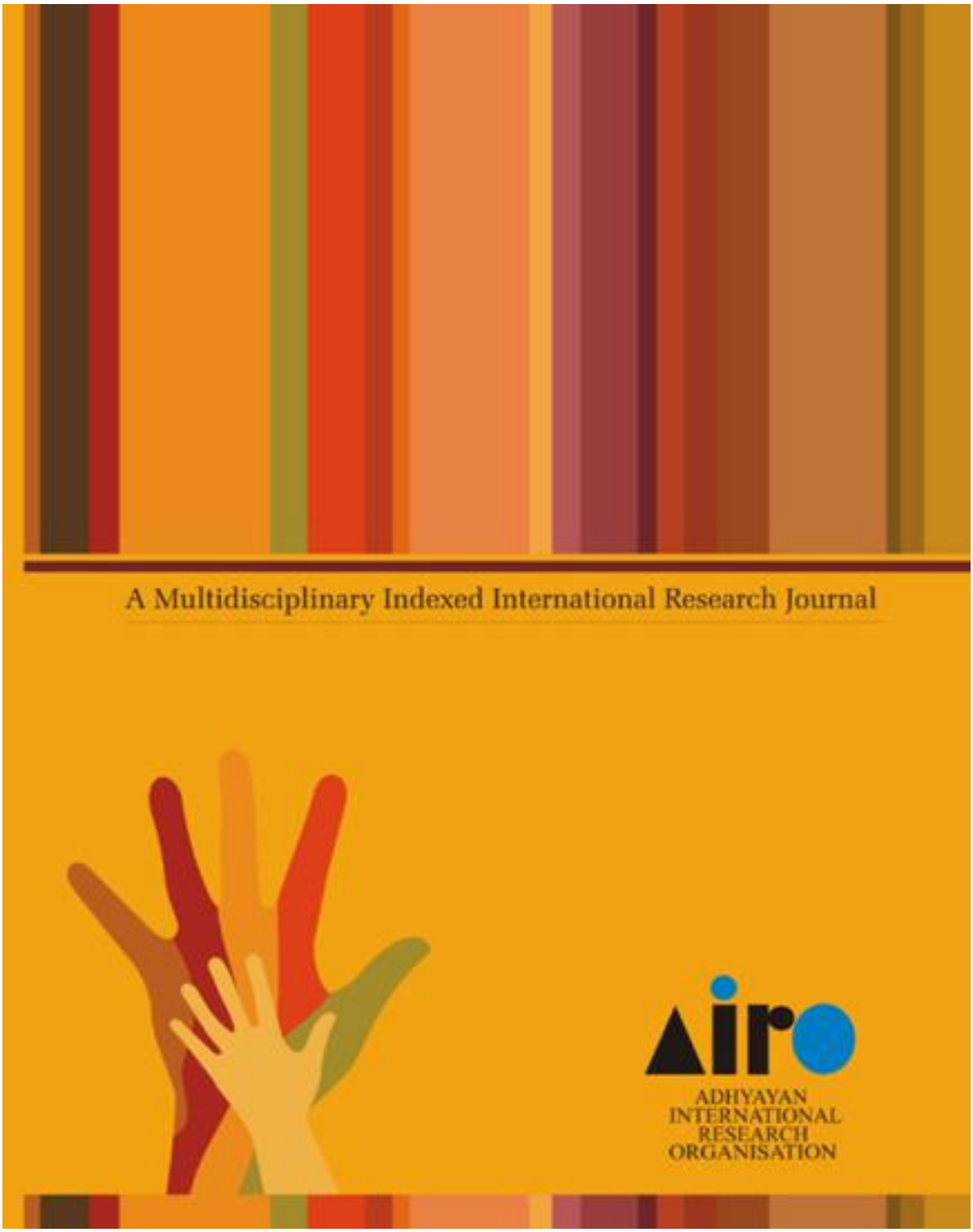
Airo International Research Journal

Volume XII, ISSN: 2320-3714
July, 2017
Impact Factor 0.75 to 3.19

ज्ञान-विज्ञान विमुक्तये

UGC Approval Number 63012



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PATTERN OF FOLIAR SENESCENCE IN LEGUMES SHOWING DIFFERENTIAL SENSITIVITY,CHANGES IN LEAF PIGMENTS, CARBOHYDRATES, REDUCING SUGARS AND NON-REDUCING AND NITROGEN IN DIFFERENT PLANT PARTS OF COWPEA (VIGNAUNQUICULATA L (WALP) AND PIGEONPEA (CAJANUSCAJAN (L.) MILLSP.) INDARKNESS INDUCED SENESCENCE

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Abstract

The loss of chlorophyll in cowpea leaf is more as compare to pigeon pea. In pigeon pea such reduction was 74, 68 and 61% in TSC, NRS, and N respectively. Minimum loss was observed in case of reducing sugars in both legumes. FW and DW were observed in leaf and stem respectively (74-75%). Reduction in FW during senescence was slow as compare to loss in DW in both the legumes. After 72hrs of darkness reduction in FW & DW was rapid. Reduction was more in cowpea than pigeon pea. Loss in TSC in cowpea was more in leaf and nodules (92 and 86%) was seen in stem. But reduction in TSC pigeon pea was more in leaf and root. The extent of decrease was 74 and 72% respectively & loss in stem TSC was only 40%. RS in cowpea parts were 8-10 mg.g-1, while in pigeon pea it was 7-13mg.g-1. Losses in RS were 80-89% in cowpea, while in pigeon pea it was 57-78%. Root and stem reduction of cowpea was more as compare to nodules and leaf. In case of pigeon pea maximum loss was observed in root and nodules. Although NRS were more in pigeon pea But losses during senescence were more in cowpea. Trends in reduction of NRS were same in both the legumes. Maximum reduction in NRS was observed in nodules followed by the leaf. Lowest changes in NRS were seen in root and stem followed by leaf. N levels were more in pigeon pea than cowpea. Nodules N were maximum and it was 10-15 times more over other plant parts. N losses during darkness induced senescence were more in nodules and root. The extent of N loss was 97-92% in cowpea and 90-86% in pigeon pea. In cowpea lowest reduction in stem N and in pigeon pea such loss was observed in leaf. The extent of losses was 64 and 75% in cowpea and pigeon pea.

Keywords: Foliar senescence, leaf pigments, carbohydrates, sugars, nitrogen,Fresh weight and Dry weight.

INTRODUCTION

Cowpea [Vignaunguiculata (L.) Walp.] Is the most important grain legume crop grown in sub Saharan Africa (Badiane et al., 2012). The value of cowpea lies in its major role in human nutrition as a protein source and also because cowpea hay is critical for feeding animals during the dry season in many parts of Africa. Cowpea is a valuable source of income for farmers and traders in West and Central Africa

(Timko et al., 2007; Diouf, 2011). Moreover, its nitrogenfixing ability is extremely valuable when used in crop rotation with cereal crops (Timko et al.,2007).According to the Food and Agriculture Organization (FAOSTAT, 2013), the top six cowpea producing countries in Africa are Nigeria, Niger, Burkina Faso, United Republic of Tanzania,Mali and Cameroon. Cowpea (Vignaunguiculata) is one of the most ancient human food sources and has probably been used since Neolithic times (Summerfield et al., 1974). One of the views suggests that cowpea was introduced from Africa to the Indian sub-continent approximately 2000 to 3500 years ago (Allen, 1983). Another view is the fact that the transversal region of the Republic of South Africa was the centre of speciation of V.unguiculata, due to the presence of most primitive wild varieties (Padulosi and Ng, 1997).Presently; cowpea is grown throughout the tropic and subtropic areas around the whole world. According to Ng (1985), during the process of evolution of V. unguiculata, there was change of growth habit, from perennial to annual growth habit and from predominantly outbreeding to inbreeding, while cultivated cowpea (subsp. unguiculata) evolved through domestication and selection of the annual wild cowpea (var. dekindtiana).Annual wild cowpea is widely distributed throughout sub-Sahara Africa.

Pigeonpea (CajanusCajan [L.] Millspaugh) is one of the major grain legume (pulse) crops of the tropics and subtropics. Pigeonpea belongs to the subtribe Cajaninae of the agriculturally most important tribe Phaseoleae under subfamily Papilionoideae of the family Fabaceae. In addition to its utilization as an annual crop, it can fit into agroforestry and shifting cultivation systems as a source of seed and forage for livestock and as a soil ameliorator. It can provide considerable residual benefit for the succeeding crops such as wheat (Johansen and others 1990). The crop has been traditionally grown as an intercrop or mixed crop with a number of cereals such as sorghum (Sorghum bicolor L.), pearl millet (Pennisetumglaucum L.), and maize (Zea mays L.). Cajanuscajan (pigeonpea) is generally observed to provide better yields than other crops in low-P soils, even without P fertilizer application, because of an extensive rooting habit and strong mycorrhizal development. Pigeonpea ranks sixth in area and production in comparison to other grain legumes such as beans, peas, and chickpeas. Its high sensitivity to salinity poses a major constraint to crop production in certain areas (Chauhan 1987). Worldwide, approximately 100 million ha of arable land are affected by salinity, which accounts for about 6–7% of the total arable area (Munns and James, 2003). In India, where 90% of the world's pigeonpea is produced, approximately 13.3 million ha of land are affected by salinity (Consortium for Unfavorable Rice Environment 2003).

Seeds germinate, grow into plants and ultimately die. Some plants live for one season, others for two, while still others live year after year. But all plants age the organs start senesce and then abscise. The best definitions of senescence are still rather vague. The most common is that senescence refers to deteriorative processes which may lead to homeostasis, which can be brought about by enzyme losses, structural disorganization and disorientation, hormonal changes, genetic shut down or combination of these (Leopold, 1975). Because of the structure and regenerative capacities of plants, it appears that individual cells, tissues and organs may undergo senescence and die without necessarily resulting in the death of whole organism. Understanding senescence as a whole is important in terms of an extended period of photosynthesis and as a result therefore more food material to mankind.

Senescence is a terminal stage of plant development. It often, but not invariably, ends in the death of cells, tissues, organs or the whole plant. At the cell level there are a number of different senescence pathways, most of which are autolytic, that is, the genetic and biochemical events originate within the senescing cell itself. Nucleus, vacuole, plastids and mitochondria interact during cell senescence. Up to the point where organelle integrity is lost, some kinds of senescence may be halted, extended or even reversed by various treatments, but beyond this threshold there is a rapid decline in viability leading to death. Developmental cell senescence and death occur during differentiation of xylem, floral tissues, embryos and seeds. Leaves, fruits and some flowers lose chlorophyll during senescence as chloroplasts differentiate into pigmented plastids. The products of Chlorophyll breakdown are deposited in the cell vacuole (Ansari et.al, 2011). Proteins and nucleic acids are hydrolysed and the nitrogen and phosphorus liberated are exported from the leaf to sink tissues. Fruit ripening shares a number of regulatory and biochemical features with leaf and flower senescence. Senescence contributes to root turnover, an important factor in global carbon balance. Plants and their parts often must attain maturity before they are able to respond to signals that induce senescence. Floral induction and seed formation stimulate senescence. In monocarpic species the entire plant undergoes reproductive death. Polycarpic plants flower repeatedly during their lifetimes, and show no clear relationship between senescence and longevity. Senescence is a strategic and tactical response to seasonal and unpredictable stresses, including changing day length, flooding, drought, excessive light, darkness, nutrient limitation and disease. The timing of senescence in relation to carbon capture and nutrient remobilization is a major determinant of crop yield. Senescence and related processes account for significant postharvest losses and food wastage.

In fact the patterns of senescence may be useful in turnover/redistribution of resources within in individual or in tissue differentiation. The well-established fact that each species of plant or animal has characteristics life span suggests that longevity may be determined genetically (Molisch, 1938). As individuals grow older, it can be seen many vital functions decline. Although the outward manifestations and the end result of senescence (which is death) are obvious; it is not precisely clear what causes these changes or how they come about; however, in many species of plants and in some animals these changes appear to be under hormonal (Sacher, 1957, 1959; Osborne, 1962, 1967, Aharoni and Liberman 1979; Richmond and Long, 1957; Naito and Iida, 1978; Addicott and Lynch, 1969; Paranjoth and Waring, 1971; and Thimann, 1981) or correlative control (Wangerman, 1965; Chibnall, 1939; Leonard, 1962 and De Pinto et.al, 2011)

Particularly crops, like wheat, rice (cereals), cowpea, soybean, pisum (legumes) face problem of lower leaf senescence, when the crop starts flowering and fruiting. The leaf senescence and accompanying breakdown of protein is an important physiological determination of yield in many agriculturally important crops. As such an understanding of the nature of enzymes involved and the genetic and physiological control of their activity will have important implications in those program aimed at increasing plant and crop productivity. In most of the legumes, N2 fixation starts to decline after flowering. Phillips (1978) has suggested that biological N2 fixation can be enhanced if plant characters may be altered to delay leaf senescence. Very few studies have been conducted on nodule senescence e.g., Alfalfa (Paau and Cowles,

1979), pea (Chen and Phillips, 1977; Develde et.al, 2006 and Kijne, 1975)Soybean (Klucas, 1977). However, no information is available on leaves and nodule senescence of cowpea, and arhar.

Therefore, the aim of the present work was todetermine leaf and nodule senescence induced by darknesseffects of salt stress in the nodules of pigeon pea. The legumes (differential sensitivity) and nodule senescence (leaf pigments, carbohydrates, reducing and non-reducing sugars and nitrogen activity) in dark induced plant and in different plant parts during senescence.

MATERIALS AND METHODS

Following experiments were carried out on the cultivar HFC-42-1 of cowpea (Vignaunquiculata L (Walp) and UPAS-120 of pigeonpea (Cajanuscajan (L.) Millsp.) The crops were raised in polythene bags in summer months for the study pattern of foliar senescence in legumes showing differential sensitivity, leaf pigments, carbohydrates, reducing sugarsand non -reducing sugars nitrogen,protein in different plant parts during darkness induced senescence and also to find out the effect of source capacity on darkness induced senescence in leaf and nodule.

Raising of plants

The seeds were treated with Biltox and sown in polythene bags of size 23x15 cm containing 2 kg of washed river sand. After germination in each bag 3 plants of uniform height were kept.

Culture solution

An equal quantity (50ml) of nitrogen free nutrient solution (Wilson andResienauer, 1963) of the composition KH_2PO_4 (2 x 10^{-3}M),K_2SO_4(2 x 10^{-3}M),$CaSO_4.2H_2O$(3 x 10^{-3}M),$MgSO_4$(1 x 10^{-3}M),$MnCl_2.3H_2O$(2 x 10^{-6}M),$ZnSO_4.7H_2O$(2 x 10^{-6}M),H_3BO_3(25 x 10^{-5}M),$CuSO_4$(5 x 10^{-7}**M**),Na_2MoO_4(5 x 10^{-7}M),Ferric citrate (1 mg/L),$CaCO_3$(1 g/l) was added to each bag at weekly intervals.

Irrigation

The bags were supplied with equal amount of a tap water daily as required except on days when nutrient solution was given.

Rhizobial inoculation

A standard, mixed Rhizobial culture solution prepared from stock froth culture, was given in equal quantity after one week of sowing.

Plant Protection Measurements

After inoculation the plants were given weekly sprays of melathion (0.1%) for the entire period of growth until the plants were shifted to darkness.

Induction of plant senescence

When plants have developed 3-4 leaves, the primary leaf was detached and the plants were shifted to a well ventilated dark room. In dark room petri disc containing potassium perchlorate, which absorbs ethylene, was placed near the bags. At this step zero hour sampling was done, subsequently the plants were sampled at 24 hours intervals. In each experiment the plants from 3 bags were removed by through washing with tap water. The plants were separated into their parts and immediately fresh weights were observed. The plant materials were dried by placing in an oven at 800C for 72 hrs. &then dry weights were taken.

For study pattern of foliar senescence in legumes showing differential sensitivity to darkness induced senescence the above treated

plants were observed for qualitative changes in leaf pigment color and shedding behavior of 1st primary leaf to judge the pattern of leaf senescence. The observations continue for 144 hrs.& after shifting the plants to dark room.For study pattern of changes in leaf pigments, carbohydrates, reducing and non-reducing sugars and nitrogen during darkness induced senescence the above treated plants were observed for quantitative changes in leaf pigment color to study the pattern of changes in leaf pigments. The observations continue for 72 hrs. after shifting the plants to dark room. At 24 hrs. Interval the plants were sampled and with the help of a cork borer, leaf discs were punched out from 1st trifoliate leaf avoiding main and lateral veins. Leaf pigments were estimated using leaf discs.

- **Leaf pigments**

From first experiment 4 leaf disc from 1st trifoliate leaf were used to extract pigments following the method of Hiscox and Israelstam (1979) by using DMSO to extract Chlorophyll from the un macerated leaf disc by keeping test tubes, containing weighed amount or leaf disc in a known volume of dimethylsulphoxide (DMSO), at 650C for 2-3 h. The Chlorophyll remains stable in DMSO and spectrophotometric readings can be taken at convenience within a day or so.

Procedure

Four leaf disc were placed in a vial containing 4 ml DMSO and Chlorophyll is extracted into fluid without grinding at 650C till the tissue became Chlorophyll free (2-4h). The liquid extract is transferred to a graduated tube and the volume made to 10 ml with DMSO. Absorbance is recorded immediately or sample-containing vials can be stored at 40C.

Determination of Chlorophyll and carotenoids content

A 3.0 ml aliquot of Chlorophyll extract is transferred to a cuvette and the absorbance (A) values are recorded at 480, 645 and 665 nm against DMSO as blank using a spectrophotometer. If the absorbance values were greater than 0.7, the extract was dilute with DMSO. Calculate the concentrations using Well burn's (1994) equations.

Chlorophyll a µg ml-1 = 12.19 A665-3.45 A645

Chlorophyll b µg ml-1 = 21.99A645-5.32A665

Carotenoids µg ml-1 = (1000A480-2.86Chla-129.9 Chl b)/221

Leaf pigments content were expressed as µg leaf disc-1 In addition to above parameters; total sugars and total nitrogen from different plant parts were investigated. At each sampling, roots, nodule, stem and leaves were separated and kept in an oven at 80°C for 48 hrs.for drying purpose. Dried plant materials were used to estimate total soluble carbohydrates, reducing and non-reducing sugars and nitrogen content.

- **Total soluble sugars**

The reducing and total soluble sugars were estimated separately in nodules, root, stem, leaves and parts. The total soluble carbohydrates were estimated by Phenol method (Dubois et al. 1956).Dried material was grind in mortar and pestle. Twenty five mg of the dried powder from each organ was extracted with water on a boiling water bath for 4-5 hours and kept overnight. Next day the extract was filtered and washed the residue thoroughly, and made a final volume of 25 ml.

Estimation of total sugars

Phenol- 2% in water&Concentrated H_2SO_4 (Analar) were used.Digestion: 1 ml of aqueous extract of above was used for estimating total sugars. To 1 ml of extract added 2 ml of 2% phenol and 5 ml of concentrated H_2SO_4. The mixture was allowed to stand for 10-20 minutes. The test tubes were shaken vigorously on a cyclomixer, cooled and O.D. was taken at 475 nm against reagent blank.Whenever necessary, the solution was diluted with concentrated H_2SO_4. Standard curve was prepared by using graded concentrations of sucrose. The results were expressed as mg sugars/g dry wt.

- **Reducing sugars:**The determination of reducing sugars of all the plant organs was done according to Nelson's method.

i. Smogy's reagent:

a) Dissolved 2 g anhydrous sodium carbonate, 16 g sodium bicarbonate,12g sodium potassium tartrate and 140 g sodium sulphate in 800 ml distilled water.

b) Dissolved 4 g $CuSO_4$ and 40 g anhydrous sodium sulphate in 200 ml of distilled water. Mixed (a) and (b) in the ratio of 4:1.

ii. Nelson's reagent:

a) Dissolved 25 g ammonium molybdatein 450 ml distilled water. To this added 21 ml of concentrated sulphuric acid.
b) Dissolved 3 g sodium hydrogen arsenate in 25 ml water with mixing.

The solution after mixing (a) and (b) was incubated at 37°C for 24 hours.

PROCEDURE

One ml of the above aqueous extract was taken and to this was added 1 ml Smogy's reagent and kept on a boiling water bath for 15 minutes. Cooled and to it added 1 ml of Nelson's reagent. Volume was made to 5 ml with distilled water. O.D. was taken at 560 nm in Beckman Spectrophotometer Model B. The standard curve was prepared using traded concentration glucose. Reducing sugars were subtracted from total soluble carbohydrates to get non-reducing sugars. Amount of total carbohydrates, reducing and non-reducing sugars are expressed on mg/g dry weight.

(4)Nitrogen content The N_2 content of different plant parts were determined separately using rapid colorimetric method of Lindner (1944).

Reagents:

1 NaOH: 1 N NaOH was prepared in distilled water

2. Nessler reagent: 100 g of mercuric iodide (HgI and 70 g of potassium iodide (KI) were dissolved in 400 ml of distilled water. 100 g of NaOH was dissolved in another 400 ml of water. The two solutions were combined and the volume was made to 1 liter with distilled water. This solution was filtered and placed for 48 hours in dark and stored in a dark brown bottle to prevent its oxidation.

Digestion

The 50 mg of powdered plant material was taken in a 50 ml conical flask to which 3 ml of 9: 1 H_2SO_4 and $HCIO_4$ mixture was added. The mixture was treated for about 25-30 minutes till the solution became colourless. The digest was diluted to 50ml with distilled water.

Estimation

The pH of the digest was adjusted to 3.0 and from this 0.5 ml of aliquot was used for estimating nitrogen. To the aliquot, 4.5 ml of In NaOH was added and thoroughly mixed. To it 0.2 ml of Nessler reagent was added drop wise with continuous shaking. The mixture was allowed to stand for 30 minutes at room temperature after which the 0.D. was determined at 450 nm against a reagent blank on Beckman spectrophotometer (Model B).A standard curve was prepared from graded concentrations of NH4Cl. The actual nitrogen values were calculated from standard curve and expressed as mg N/g dry weight.

For study pattern of changes in total carbohydrates, reducing and non -reducing sugars and nitrogen in different plant parts during darkness induced senescence.

The dried nodules were grinded and used to estimate the above explained parameters like total soluble carbohydrates, reducing and non-reducing sugars and nitrogen contents.In addition to above parameters, fresh and dry weights of different plant parts, total sugars, reducing and non-reducing sugars and total nitrogen from different plant parts (nodule, root, stem and leaf) were estimated as described. The decrease in various parameters were further calculated as % decrease over control and presented in tables.

RESULTS AND DISCUSSION

For study pattern of foliar senescence in legumes showing differential sensitivity to darkness induced senescence.The above treated plants were observed for qualitative changes in leaf pigment color and shedding behavior of 1st trifoliate leaf to judge the pattern of leaf senescence. The observations continue for 144 hrs. after shifting the plants to dark room. Senescence and shedding pattern of 1st trifoliate leaf is shown in table1. The observation period was extended to 144 hrs. after shifting the plants in darkness. The data indicated fast loss in green colour of cowpea leaf as compare to pigeon pea. Fifty and 100% senescence in cowpea was achieved in 3 and 5 days of darkness. Similarly leaf shedding was initiated on 4th days of observation and it was completed by 7th days of darkness. In case of pigeon pea loss in green colour was slow. Fifty and 100% colour change was seen after 3rd and 6th days of observation. However, initiation of leaf shedding was obtained on 5th days and upto 7th days of darkness induced 75% leaf shedding. Therefore, to avoid shedding

Table - 1 Senescence and shedding pattern in 1st trifoliate leaf of cowpea and pigeon pea

Sr. No.	Observation time (hrs.)	Cowpea		Pigeon pea	
		Senescence *	Shedding**	Senescence *	Shedding**
1.	0	0.0	0.0	0.0	0.0
2.	24	25.0	0.0	20.0	0.0
3.	48	50.0	0.0	40.0	0.0
4.	72	75.0	25.0	60.0	0.0
5.	96	100.0	50.0	80.0	25.0
6.	120	100.0	75.0	100.0	50.0
7.	144	100.0	100.0	100.0	75.0

* Senescence = Approximate % fading of 1st trifoliate leaf color

** Shedding =Approximate % shedding of 1st trifoliate leaf

process, observation was restricted to 72 hrs. of darkness in all further experiments.

For study pattern of changes in leaf pigments, carbohydrates, reducing and non-reducing sugars and nitrogen during darkness induced senescence.

Changes in leaf pigments like chlorophyll a, b, total chlorophyll and carotenoids, total soluble carbohydrates, reducing and non-reducing sugars and nitrogen in 1st trifoliate leaf of cowpea and pigeon pea are shown in table2. Leaf pigments were more in leaf disc of pigeon pea as compare to cowpea. Initial chlorophyll a, b, and carotenoids in pigeon pea were 22, 33 and 28% more over cowpea. Reduction in these pigments was high in cowpea as compare to pigeon pea. Decrease in chlorophyll a during darkness induced in leaf senescence was more over chlorophyll b in cowpea. The extent of reduction was 93 and 66% in chlorophyll a and b respectively. In case of pigeon pea such reductions in chlorophyll a and b were 55 and 59% respectively. Total chlorophyll loss was 85% in cowpea as compare to 56% in pigeon pea. Loss in chlorophyll was variable at different samplings. Maximum decrease was observed at lost sampling. During leaf senescence carotenoids accumulated in both legumes. However, such accumulation in carotenoids was also more in cowpea over pigeon pea. Extent of carotenoids accumulation was 111 and 76% in cowpea and pigeon pea.

Various fractions of carbohydrates and nitrogen reduced in both the legumes. Amount of these nutrients were more in pigeon pea. Reduction in non-reducing sugars was maximum (86%) followed by total soluble carbohydrates and nitrogen (80% in both) in case of cowpea. In pigeon pea such reduction was 74, 68 and 61% in TSC NRS, and N respectively. Minimum decrease was observed in reducing sugars in both the legumes.

Table -2 Effect of dark induced senescence on biochemical parameters (µg. leaf disc-1) in 1st trifoliate leaf of cowpea and pigeon pea

Biochemical parameters	C O W P E A					P I G E O N P E A				
	Sampling time(hrs.) after darkness 0	24	48	72	% increase over control	Sampling time (hrs.)after dark period 0	24	48	72	% increase over control
Chlorophyll a	58	53	14	4	-93	71	65	54	32	-55
Chlorophyll b	24	20	16	8	-66	32	28	22	13	-59
Total Chl.	82	73	30	12	-85	103	93	76	45	-56
Carotenoids	28	35	40	59	+111	36	45	51	64	+76
Total soluble sugars	72	75	30	14	-80	81	86	40	26	-68
Reducing sugars	14	12	8	6	-57	15	13	10	9	-53
Non reducing sugars	58	63	22	8	-86	66	73	30	17	-74
Nitrogen	10	8	5	2	-80	13	10	7	4	-61

Fresh (F) and Dry (D) weight (W) of various plant parts during 144 hrs. of darkness are given in table 3 and 4. FW and DW of different plant parts like root, nodules and stem were higher in pigeon pea. However, Leaf FW and DW was more in cowpea. In cowpea leaf has maximum FW and nodules have minimum FW. However, maximum DW was observed in root and minimum DW was in nodules. In pigeon pea. Maximum FW and DW were seen in root and minimum in nodules. In cowpea maximum reduction in FW of leaf (92%) was followed by nodules (83%) and minimum decrease in FW was observed in stem (76%) Similar trends in loss in DW were observed in these plant parts. Extent of DW loss during darkness induced senescence was 87-76% of the initial values. In pigeon pea loss in FW and DW was maximum in root 90 and 85% respectively. Minimum reduction in FW and DW was observed in leaf and stem respectively (74-75%) Reduction in FW during senescence was slow as compare to loss in DW in both the legumes. After 72hrs. of darkness reduction in FW & DW was rapid.

For the study pattern of changes in total soluble carbohydrates, reducing and non-reducing sugars and nitrogen in different plant parts during darkness induced senescence.

Data on various biochemical estimation in plant parts have been given in table 5 and 6. Total soluble carbohydrates (TSC), reducing sugars (RS), non-reducing sugars (NRS) and nitrogen (N) in all plant parts were more in pigeon pea as compare to cowpea. RS was low in all plant parts of both the legumes over other metabolites. Nodules were having highest level of these metabolites as compare to other plant parts. On the other hand roots were having lowest level of various metabolites, except RS. All metabolites reduced during senescence. Reduction was more in cowpea as compare to pigeon pea. Loss in TSC in cowpea was more in leaf and nodules (92 and 86%) was seen in stem. Reduction in TSC in pigeon pea was more in leaf and root. The extent of decrease was 74 and 72% respectively. Loss in stem TSC was only 40%. RS in cowpea parts were 8-10 mg.g-1, while in pigeon pea it was 7-13mg.g-1. Losses in RS were 80-89% in cowpea, while in pigeon pea it was 57-78%. Reduction in root and stem of cowpea was more as

Table – 3 Fresh and dry weights (mg. plant parts-1) of plant parts of cowpea during darkness induced senescence

Parameters	Plant parts	Sampling time(hrs.) after shifting to dark room 0	24	48	72	96	120	144	% increase over control
Fresh weight	Root	1135	1204	861	791	522	361	204	-80
	Nodules	974	783	677	515	336	216	166	-83
	Stem	1337	1142	986	808	709	548	319	-76
	Leaves	1426	1097	888	693	296	110	0	-92
	Total	4872	4226	3412	2807	1863	1237	689	-86
Dry weight	Root	297	169	98	83	76	47	42	-86
	Nodules	199	88	72	64	48	32	25	-87
	Stem	278	156	117	96	83	75	67	-76
	Leaves	263	173	94	81	42	34	0	-87
	Total	1037	586	391	324	249	188	134	-87

Airo International Research Journal

Volume XII, ISSN: 2320-3714
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Impact Factor 0.75 to 3.19

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UGC Approval Number 63012

Table – 4 Fresh and dry weights (mg. plant parts-1) of plant parts of pigeon pea during darkness induced senescence

Parameters	Plant parts	Sampling time(hrs.) after shifting to dark room 0	24	48	72	96	120	144	% increase over control
Fresh weight	Root	1671	998	823	718	478	309	161	-90
	Nodules	993	874	786	567	386	269	217	-78
	Stem	1415	1218	1038	979	854	677	449	-68
	Leaves	1378	986	817	768	648	458	362	-74
	Total	5057	4076	3464	3032	2366	1713	1189	-76
Dry weight	Root	366	275	163	107	93	65	54	-85
	Nodules	217	186	109	86	74	58	46	-79
	Stem	298	214	184	111	89	80	74	-75
	Leaves	256	208	177	113	76	63	51	-80
	Total	1137	883	633	417	332	266	225	-80

As compare to nodules and leaf. In case of pigeon pea maximum loss was observed in root and nodules. Although NRS were more in pigeon pea. But losses during senescence were more in cowpea. Trends in reduction of NRS were same in both the legumes. Maximum reduction in NRS was observed in nodules followed by leaf. Lowest changes in NRS were seen in root and stem followed by leaf. Lowest changes in NRS were seen in root and stem of both the legumes. N levels were more in pigeon pea over cowpea. Nodules N were maximum and it was 10-15 times more over other plant parts. N losses during darkness induced senescence were more in nodules and root. The extent of N loss was 97-92% in cowpea and 90-86% in pigeon pea. In cowpea lowest reduction in stem N and in pigeon pea such loss was observed in leaf. The extent of losses was 64 and 75% in cowpea and pigeon pea respectively.

DISCUSSION

Biological systems are dynamic systems which run on the basis of turnover of constituents. Homeostasis establishment and evolution also are based on turnover of organisms and groups of organisms. Because of the structure and regenerative capacities of plants, it appears that individual cells, tissues and organs may undergo senescence and die without necessarily resulting in the death of whole organism.In fact the patterns of senescence may be useful in turnover/redistribution of resources within in individual or in tissue differentiation.

In senescence the various hydrolytic activities are increased and synthetic processes are diminished ultimately leading to the death of the plant. The senescence is beneficial to the plant in a sense that from older tissue reallocation of vital metabolites occurs and undeserved organs are eliminated from the parent plant. Understanding of senescence can be beneficial in many crop plants, if it can be regulated after flowering either by the spray of nutrients or hormones which may be limiting in the plants at that stage. In legumes N fixation is an important process through which free N of atmosphere is fixed for the utilization of plants and remaining fixed N is added into the soil to improve fertility. N2 fixation declines in most of the legumes after flowering and it has been reported that 60-70%

N2ase activity (Swaraj, et.al, 1994; Sunita and Swaraj, 1996 and Swaraj, et.al, 2003.) is reduced in gram after flowering..

Particularly crops, like wheat, rice (cereals), cowpea, soybean, pisum (legumes) face problem of lower leaf senescence, when the crop starts flowering and fruiting. The leaf senescence and accompanying breakdown of protein is an important physiological determination of yield in many agriculturally important crops. In most of the legumes, N2 fixation start to decline after flowering. Phillips (1978) has suggested that biological N2 fixation can be enhanced if plant characters may be altered to delay leaf senescence.

Following experiments were carried out on the cultivar HFC-42-1 of cowpea (Vignaunquiculata L (Walp) and UPAS-120 of pigeon pea (Cajanuscajan (L.) Millsp.) The crops were raised in polythene bags in summer months. In all these experiments leaf and nodule senescence was induced by keeping the plants in dark.

The above treated plants were observed for qualitative changes in leaf pigment color and shedding behavior of 1st primary leaf to judge the pattern of leaf senescence. The observations continue for 144 hrs. After shifting the plants to dark room.The results revealed rapid loss in green color of cowpea as compare to pigeon pea leaves. Rate of leaf senescence also showed similar trends especially for 100%. In cowpea it was early by a day. Thus it is clear that chlorophyll loss in cowpea was faster than in pigeon pea. It can be due to stable chloroplast and slow rate of chlorophyllase. Differential degradation of chlorophylls has been observed in normal and mutant of much plant. Similarly leaf shading was also initiated a day early in cowpea. On prolonging darkness period, leaf shedding in cowpea was 100% by 7th day; however, leaf shedding in pigeon pea was 75% only (table-1). Therefore these two legumes were having differential rate of senescence and abscission.

These plants were observed for quantitative changes in leaf pigment color to study the pattern of changes in leaf pigments. The observations continue for 72 hrs. After shifting the plants to dark room. At 24 hrs. Interval the plants were sampled and with the help of a cork borer, leaf discs were punched out from 1st trifoliate leaf avoiding main and lateral veins. Leaf pigments were estimated using leaf discs.

Above differential rate of leaf senescence between cowpea andpigeon pea leaves were further investigated using various morpho-physiological parameters. It is apparent from table - 2 that chlorophyll a, b and carotenoids were more in pigeon pea as compare to cowpea. Since chlorophyll a and b were more in pigeon pea, therefore if we assume uniform rate of chlorophyll degradation in both the legumes, even more chlorophyll remained undegraded due to higher initial content inpigeon pea. Similarly higher content of chlorophyll a over b in both the legumes was observed. Degradation of chlorophyll a was rapid as compare to chlorophyll b. Due to initial higher content of chlorophyll a, more amount of this may remain undegraded. How chlorophyll a and b are differentially degraded? It is not well understood even now. During darkness induced leaf senescence, more losses (93 and 66%) in chlorophyll a and b in cowpea were observed as compare to pigeon pea (55 and 59%). Less degradation of chlorophyll in pigeon pea may be due to deeply embedded pigments in chloroplast matrix. 2nd possible explanation of differential degradation of chlorophylls pigments may be slow release of chloropllase from the vacuoles or differential rate of chlorophyllase activity in these two legumes.

Carotenoids were also more in pigeon pea. However, during senescence carotenoids accumulatedmore in cowpea as compare to pigeon pea. Thus cowpea leaf appeared more yellow and also early over pigeon pea. Accumulation of carotenoids during senescence may be due to less degradation or higher synthesis of carotenoids. In cowpea leaf more net accumulation of carotenoids may be due to their slow metabolism.

Various fractions of carbohydrates were also more in pigeon pea. Higher content of TSC, RS and NRS in pigeon pea leaf can be due to more photosynthetic rate. Similarly leaf N in pigeon pea was also more probably due to high rate of nitrogen fixation in root nodules as compare to cowpea. During darkness induced leaf senescence all these nutrient levels reduced. Such decrease is due high utilization of these nutrients for maintenance respirationand less synthesis in absence of light. Depletion of NRS was higher followed by TSC. Minimum loss in RS was observed during senescence. It is understandable because during respiration aoptimum level of RS is to be maintained, otherwise respiration will decrease leading to death of plant organ. Losses in leaf N during senescence is due to decreased transport of fixed N, which is very rapidly reduced under darkness, from root. N and DW of different plant parts like root, nodule stem and leaf after 144 hr. of darkness are presented in table - 3 and 4. The values of FW and DW were higher in pigeon pea, except leaf FW and DW which were more in cowpea.Distribution of FW and DW in different plant parts depend upon resource production capacity i.e., source efficiency of these processes.

During senescence FW and DW in different plant parts were reduced. Extent of losses in both the legumes was almost comparable with little marginal differences only. Losses in FW and DW in different plant parts were not uniform. Roots were showing maximum losses as compare to other parts. Reduction in FW of plant parts can be due to less absorption of water from the medium and its translocation to other plant parts. However losses in DW during senescence can be explained on the basis of increased respiration. Thus it seems that losses in FW and DW of plant parts of both the legumes are after effects of senescence rather than the cause of senescence.The dried plant materials were grinded and used to estimate the above explained parameters like total soluble carbohydrates, reducing and non-reducing sugars and nitrogen contents.Result of TSC, R.S, NRS, and N in different plant parts have been shown in table - 5 and 6. The nutrients were more in pigeon pea as compare to cowpea. Higher level of nutrients in pigeon pea indicates more efficient photosynthesis and nitrogen fixation. Low level of RS in all plant parts amongst various fraction of carbohydrates may be due to rapid utility of RS in metabolism and immediate replenishment by hydrolysis of carbohydrates to maintain a optimum pool of RS. Nodules have highest level of these metabolites indicating high energy requirement for nitrogen fixation. Roots have been shown to contain lowest level of various metabolites suggests translocating nature and poor storage capacity of root. During senescence all these metabolites decreased. Such depletion of nutrients was more in cowpea. Losses in TSC in leaf and nodule were more than 90% of the initial values. Under darkness CO_2 fixation through dark reaction of photosynthesis is completely prevented and residual TSC are immediately translocated to nodules to maintain nitrogen fixation. These are immediately used in nodule and on further prolongation of darkness nitrogen fixation is greatly reduced. Similarly nodule N also depleted more than 90% in cowpea and more

than 80% in pigeon pea. Depletion of nodule N during darkness induced senescence can be explained on the basis of loss of nitrogen fixation and utilization of residual nitrogen by root and shoot tips so that immediately after shifting to lightness regrowth of all plant parts can be rejuvenated. This response depends upon certain critical period of darkness before that the whole steps of senescence can be reversed. However after this period senescence is irreversible. Generally this critical period of darkness in legumes is 3-4 days only.

When the plants were exposed to darkness, there was rapid decrease in chlorophyll content. However, the decrease in chlorophyll content was more drastic at 48 hrs.than at 24-72 hrs. The increase in carotenoids indicates that it may be a masking effect of chlorophylls, i.e., as chlorophylls decreased during senescence, carotenoids appear. But it is actual synthesis as well as degradation of carotenoids. The carotenoids are also equally susceptible to senescence as that of chlorophylls. In literature degradation of chlorophyll has been a well-documented parameter of senescence (Goldthwaite and Lae-tsch, 1967; Back and Richmond, 1971)

However, very little information is available on the fate of carotenoids during senescence. The exact mechanism of chlorophyll degradation during senescence has not been worked out in detail but photo-oxidation is completely ruled out. The enzyme chlorophyllase probably function predominantly in regulating chlorophyll synthesis as well as degradation of chlorophyll.Although, It has been reported that the synthetic and hydrolytic function are attributed to two or more separate chlorophyllases (Choe and Thimann, 1974). On the other hand, Holden (1974) found no consistent relationship between leaf age and chlorophyll in a number of species and the activity of chlorophyllase in Raphanus leaves declines during senescence (Phillips and Fletcher, 1969). However, when in a soybean population that maintained its chlorophyll, RUBP Carboxylase activity and N fixation activity in root nodules throughout their development showed delayed senescence (Abushakara et al., 1978).

Decrease in chlorophyll is well correlated with decrease in nitrogenase activity and the assumption that nodules act as powerful sink for photosynthates from the leaves is probably not true in cowpea because the roots were detached under water and the shoots are kept in darkness, the normal pattern of degradation of plant pigments has been observed. Even if actively growing tips are also removed the leaves undergo normal senescence (Goswami, 1982 unpublished). Thus, indicating that the degradation of plant pigment has nothing to do with the N- fixation. It may be possible that the transport of cytokinins for which roots are the active sites is inhibited under darkness. In many cereals the carotenoids have been found to be more stable in green leaves but when etiolated oat leaves undergo senescence in dark, the carotenoids are lost at about the same rate as that in green leaves of the same age (Tetley and Thimann, 1975). The possibility that degraded products of carotenoid can be converted into ABA cannot be ruled out. It may be possible that the rapid degradation of carotenoids may lead to accumulation of ABA in leaves which ultimately resulted in the degradation of chlorophyll (Aharoni and Richmond, 1978). Nothing is known about the enzymology of chlorophyll degradation.

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